Demonstration of Cost Effective Marker Assisted Selection for Biomass Yield in Red Clover (*Trifolium pratense* L.) – Part 1: Paternity Testing.

Heathcliffe Riday
U.S. Dairy Forage Research Center (USDA-ARS), Madison, WI 53706

Many methods have been proposed to incorporate molecular markers into breeding programs. Presented is a cost effective marker assisted selection (MAS) methodology that utilizes individual plant phenotypes, seed production-based knowledge of maternity, and molecular marker-determined paternity. Progeny of 20 maternal halfsib families, each from three 96 parent polycrosses, were established in replicated multi-environment space plant evaluation nurseries in 2008. Plant vigor was visually estimated (Riday, 2009) for approximately 5 separate harvests at each nursery during the 2008 and 2009 growing seasons. Plant tissue was collected from all parental plants and each plant evaluated in the nurseries. DNA was extracted from all collected plant tissue and 11 SSR markers were amplified from each DNA sample (Riday and Krohn, 2010). Using known maternal information and SSR information, paternity of progeny genotype was determined with CERVUS statistical software package. A simple 100 permutation validation approach was used to demonstrate selection gains. 80% of progeny plants with genotypic and phenotypic information were used to rank maternal and paternal halfsib families. Rankings from the 80% estimation sets were used to rank 20% validation sets based on a progeny's maternal and paternal parent. Positive and negative selection was practiced for the full range of selection intensities on the validation sets with average selection gains determined across all 100 20% validation sets. In all three populations tested, selection gains were greatly increased with molecular marker based paternal information (Fig. 1). Surprisingly, selection on the paternal parent had much greater gains. This MAS methodology is non-linkage based, meaning any co-dominant molecular markers from any genomic location can be used. This MAS method can be implemented using current statistical software packages in any diploid or allopolyploid with at least one diploid genome and accompanying genome specific molecular markers.

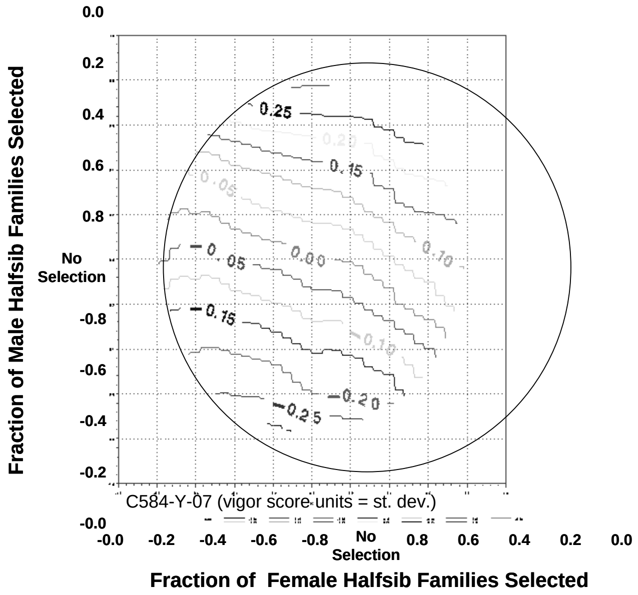

Fig. 1 Paternal and maternal selection gains for vigor in red clover (Trifolium pratense L.) population C584-Y-07.

Riday, H. 2009. Correlations between visual biomass scores and forage yield in space planted red clover (*Trifolium pratense* L.) breeding nurseries. Euphytica 170:339-345.

Riday, H. and A.L. Krohn. 2010. Genetic map-based location of the red clover (*Trifolium pratense* L.) gametophytic self-incompatibility locus. Theor Appl Genet [Epub ahead of print].